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EVIDENCE OF MULTIPLE FACTORS IN MICE AND RATS

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THE object of this paper is to record certain data on the inheritance of two complex characters and analyze these data together with those obtained in certain analogous experiments by other investigators. This is done with a view to ascertaining what they contribute to our knowledge of the relative merits of the two more or less contradictory hypotheses, multiple segregating factors or a single fluctuating factor which are being advocated by geneticists to explain certain cases of inheritance.

It will be useful at the outset to state in a somewhat definite manner how the alternative hypotheses differ from one another. MacDowell (1916) in a recent paper has clearly and precisely defined the two views. From this start the following statement may be made. The first view supposes that variations of the germ plasm are in the nature of fluctuations. The germ plasm is in a continuous state of variation. The hereditary characters all vary under observation and this is taken to mean that the factors in the germ plasm determining them also vary. Fluctuation in the character is measured and used as a means of detecting and recording a similar though not identical variation in the germinal factor underlying and determining the character under observation. To use a concrete example. In a given species the individuals are of various sizes, some larger, some smaller. Some of this variation is considered as in part due to non-heritable environmental influences. There is, however, a distinct ''inheritance'' of size. This is considered the result of a variable germinal factor which as Castle suggests may be ''Perhaps some substance or ferment which varies in amount, larger amounts producing larger results'' (1916, p. 55). In crosses between animals possessing distinctly different degrees of the size ''factor'' blending rather than alternative inheritance is supposed to result. This also holds in the case of crosses between races differing only slightly in the degree of the size character. In such cases says Castle ''we do not find it easy to detect segregation'' (1916, p. 55).[1]

The ''second'' view supposes that complex physiological results such as the ''size'' of a certain animal depend upon a complex of genetic factors. All these factors are concerned in the growth and therefore the size of the animal. The germinal determiners of these many factors are considered to be units.[2] As such their behavior in inheritance is definite, non-blending, and essentially Mendelian in nature. The blending, fluctuating nature of the character studied is supposed to be due to environmental factors, to the large number of hereditary factors involved, and to absence or incompleteness of dominance.

With this rather incomplete statement we may turn to an examination of the experimental data.

I. The Inheritance of Spotting in Mice

Experiments on the inheritance of spotting in mice have been carried on by the writer since 1909. As they progressed it became evident that spotting in mice offered remarkable material for the investigation of the inheritance of a variable character. Spotting as a character is easily measurable and classifiable. It is not affected by changes in the external environment. Variations in degree of spotting are almost certain to be quickly recognized.

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[1] It is interesting to note in this connection, the quotation from Castle given by MacDowell, 1916, page 741, describing the inheritance of minute quantitative differences in intensity of yellow pigmentation in guinea-pigs. The later work of Wright, 1915, also has a distinct bearing on this particular case in that it shows four truly allelomorphic grades of intensity of pigmentation in guinea-pigs. Fluctuation occurs about these four variation centers and Castle's ''complete series of intermediates'' between red and cream color in guinea-pigs is broken into three distinct centers of variation with overlapping range.

[2] East, 1912, has made clear the fact that variation within the unit, under certain conditions must of course be supposed to be possible.

In mice a so-called recessive "piebald" spotting has long been known. In addition a distinctly different hereditary type, commonly dominant, was recorded by Miss Durham (1911), and was further investigated by the writer who found it to be entirely independent in inheritance from the "piebald" type. This "dominant" spotting I have called the "black-eyed white" type of spotting. Further in 1914 a third type of spotting known as "blaze," consisting primarily of a white forehead spot, was reported on by the writer. This type has since been found to be independent of the other two in inheritance. The existence of at least three genetically distinct types of spotting in mice is therefore proven. It is not the purpose of this paper to generalize from this fact, *but to attempt to show that various grades of a single type of spotting may be introduced into a cross and may reappear in the F_2 or back-cross generation, not rarely, but in a considerable proportion of the animals obtained.* This is a common breeding test of segregation as compared with contamination and of alternative as compared with blending inheritance.

The races of mice used offer for the most part extremely homogeneous material. The experimental animals come from closely inbred races which have undoubtedly reached a degree of genetic homogeneity which would lead to definite results in breeding, and a clearer opportunity to observe segregation if it existed. The degree of white spotting in the races of piebald mice used is estimated by recording the approximate per cent. of the dorsal surface which is pigmented. This method is subject, as is any system of arbitrary grades, to a certain amount of error. It is, however, reasonably accurate and affords a fair measure of the degree of pigmentation of any individual.

The first experiment to be recorded is a cross involving an English piebald race descended from black-eyed white mice (Little, 1915). These piebalds vary in the extent of dorsal pigmentation from 20 to 96 per cent. It will be seen from Table I that there are essentially two modes to the variation curve, one of these occurring at 30 to 44 per cent., and the other at from 80 to 92 per cent. Animals from this race were crossed with dilute brown self mice, and with mice from a yellow and from a mixed black-agouti and black race. The F_1 generation consisted of solid colored young. When these F_1 self young were crossed back with animals from the piebald race, a range of variation was obtained which is shown in the second line of Table I.

TABLE I

Percentage of Dorsal Pigmentation

	0–4	5–8	9–12	13–16	17–20	21–24	25–28	29–32	33–36	37–40	41–44	45–48	49–52	53–56	57–60	61–64	65–68	69–72	73–76	77–80	81–84	85–88	89–92	93–96
Piebald English stock..	—	—	—	—	2	2	11	27	18	26	18	9	14	6	10	4	12	2	7	17	13	16	15	1
Back-cross piebald....	—	—	—	—	—	—	1	1	6	13	6	7	10	2	11	2	6	1	3	5	6	10	10	5

It will be seen that even though the total of the young obtained is less than in the pure piebald race, its range of variability is essentially the same and there are only four animals among those raised from the pure piebald race which fall outside of the range of variation of the back-cross generation. There is, moreover, no evidence of a single mode in the curve of the back-cross generation, but the modal centers of the parent piebald race are each represented by a large number of young in the back-cross generation.

Another cross in which larger numbers have been recorded is one between Japanese spotted mice and two races of self pigmented mice raised at the Bussey Institution. About two years before the self mice were crossed with the Japanese, certain spotted mice of common ancestry with them were separated off as a different stock. The range of variation in the degree of spotting within this last named race may be considered a fair indication of the probable appearance of the self animals used if they had been spotted. It will be seen from the first row of Table II that, in this race, animals varying from 56 to 96 per cent. were obtained. There is a tendency for a mode to be formed between 80 and 92 per cent. The Japanese race used is one which for several years has been closely inbred and which holds remarkably true to type. Its range of variation is shown in the second row of Table II. It will be seen that the animals possess from 4 to 36 per cent. of the dorsal surface pigmented. A distinct mode is observed between 13 and 16 per cent.

TABLE II

Percentage of Dorsal Pigmentation

	0–4	5–8	9–12	13–16	17–20	21–24	25–28	29–32	33–36	37–40	41–44	45–48	49–52	53–56	57–60	61–64	65–68	69–72	73–76	77–80	81–84	85–88	89–92	93–96
Tame race...	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	6	10	5	13	11	10	19	9
Japanese spotted....	1	8	74	92	65	23	7	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F₂ spotted...	—	—	—	—	3	2	5	6	4	10	6	5	9	3	11	5	6	4	8	10	8	9	14	18
Back-cross spotted Gen. 1....	—	—	3	2	4	4	14	14	14	16	5	13	8	7	4	1	6	3	—	2	1	2	5	8
Back-cross spotted Gen. 2....	—	1	1	1	—	—	1	1	—	5	4	6	5	1	3	1	—	1	—	1	—	—	3	3

The F_1 generation obtained from crossing the Japanese race with the self dilute brown or brown agouti animals above referred to, consists of solid colored animals and is therefore not to be recorded. The F_2 generation in which 146 piebald animals have been recorded contains mice ranging from 20 to 96 per cent. pigmented. Among the 146 animals recorded, 20 or 13.6 per cent. fall in grades characteristic of the Japanese grandparents. 94 or 63.6 per cent. are of grades found in spotted animals of the other grandparental race, see Table II, line 3. Two other generations of great interest have been tabulated. The first of these, line 4, Table II, is the result of back crossing F_1 generation animals with animals of the Japanese parent race. In this generation there is distinct evidence of segregation. Fifty-five of the 131 animals recorded fall within the limits of the Japanese grandparental race and 35 within the variation limits of the other grandparental spotted ancestor, and of these an appreciable number occur at the upper limit of variability, there being no evidence of a tapering of the curve at this point. This fact would appear to be of marked significance. When first generation back-cross animals are bred *inter se,* a second back-cross generation is produced, which, though it includes only 38 animals, has given extremely interesting results, see line 5, Table II. There are five animals which are distinctly Japanese segregates, and of these three show a degree of pigmentation which would make them easily mistaken for even the extreme variants in the Japanese race. At the other end of the curve it is interesting to note that six of the thirteen young reproducing the condition of the non-Japanese grandparental stock, fall into the two extreme upper classes and may be considered as true segregates rather than due to any chance occurrence of an abnormal physiological condition.

A third cross involving spotted mice has been made. This is between animals showing a small white forehead spot and a self race. The dilute brown self race used for one parent is the same that has been already recorded above in the second experiment with mice. The range of variation of white spotting in the blaze or forehead spot race used, is recorded in line 1 of Table III. The grades of spotting designated in Fig. 1, numbers 1 to 4, represent the increasing degrees of white spotting. The animals comprising this spotted race are all of them pure

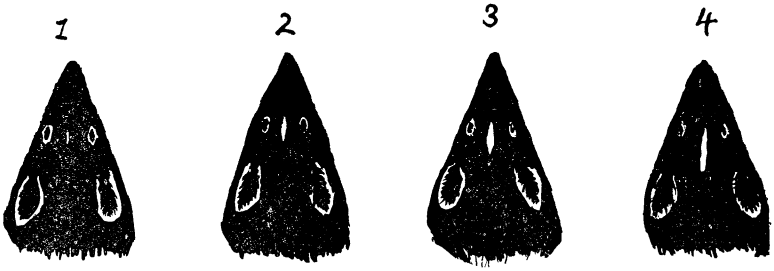

FIG. 1

wild mice descended from a few individuals captured at Forest Hills, and at Wenham, Mass. The F_1 generation obtained by crossing the "blaze" and dilute brown self races is all self in character. The F_2 generation shows a distribution of young recorded in line 2, Table III.

TABLE III

GRADE OF SPOTTING

	−½	1	1½	2	2½	3	3½	4
Pure blaze animals	7	21	4	20	6	5	3	2
F_2 blaze animals	3	15	1	16	10	7	3	1
F_3, F_4, F_5 blaze animals	25	136	24	107	25	55	7	11

It will be seen that the extremes of variability in F_2 are the same as in the pure inbred wild blaze race. F_3, F_4, and F_5 animals raised *without selection* give a result recorded in line 3 of Table II, and again show the same limits of variation. It will be seen, therefore, that the blaze character is segregated from the cross without apparent modification, although the extremely minute degrees of spotting which it includes are those which one might expect would be modified or entirely swamped if contamination between the gametes of the self and blaze races occurred. This cross together with the cross between mutant and wild rats recorded by Castle to be considered later, give conclusive evidence that even a minute quantitative character segregates after crossing and does not afford grounds for the objection raised by Castle that crosses between races differing slightly in size or like fluctuating characters do not readily show segregation.

To sum up the results of spotting inheritance in mice, it may be said that all the crosses made show a reappearance of grandparental conditions in F_2, back-cross, and other advanced hybrid generations. The reappearance of these grandparental types is frequent enough to lead one to conclude that if segregation in a strictly Mendelian sense is not taking place, that at least the outward appearances of such a process are all of them present.

II. The Inheritance of Spotting in Rats

Castle's work on the inheritance of fluctuations in the hooded coat pattern of rats is well known to all geneticists, and may now be considered in an attempt to examine the bearing of spotting on the inheritance of fluctuating characters in general. The hooded rats with which Castle worked have been shown clearly by Doncaster, Mudge, and others, as well as by Castle himself, to be recessive to self or solid colored coat in inheritance. The hooded pattern is, however, subject to wide fluctuation producing a series of rats from those with the whole dorsal surface and most of the ventral surface pigmented,

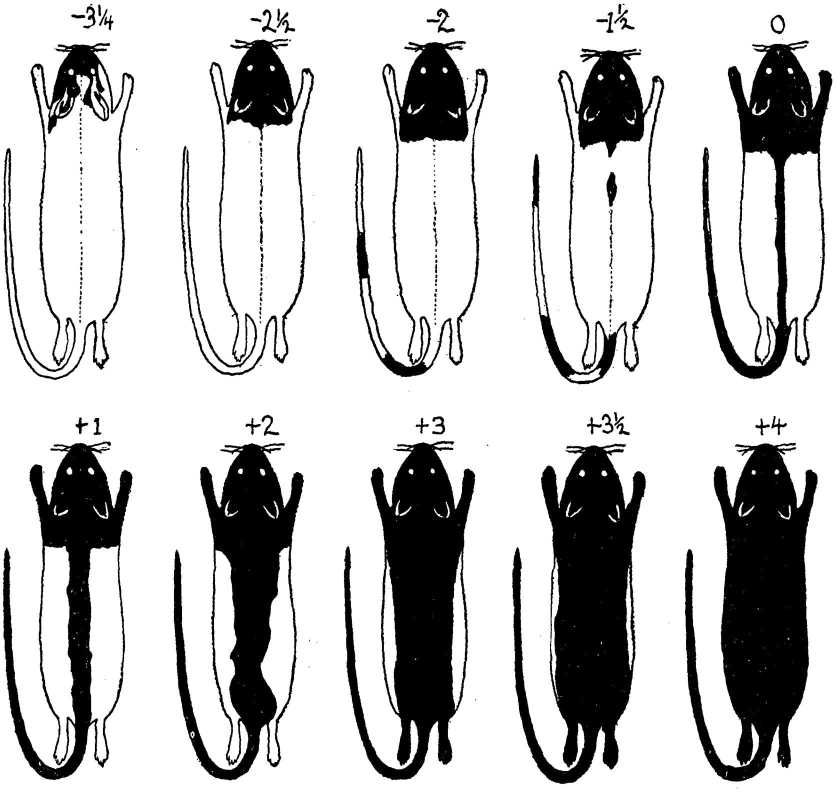

Fig. 2

In grades higher than + 4 the whole dorsal surface is pigmented and the ventral surface is becoming progressively more so.

to rats in which only small spots around the eyes remain pigmented. The following diagram will show roughly the limits of variation and some of the intermediate types as graded by Castle. Throughout the course of his selection experiments, Castle has at times crossed the plus variants with the minus variants or else crossed selected animals from the plus or minus series with wild or with Irish rats. Both the last mentioned varieties are essentially self pigmented animals and are dominant in inheritance to the hooded pattern. The race with which Castle started his selection experiments showed within the first two generations of selection rats varying from grade minus two to grade plus three and three quarters.

Castle and Phillips, 1914, have recorded the results of two crosses between minus variants and plus variants. The first of these crosses was between females of minus two grade in the sixth selection generation and males of plus three and one half or three and three quarters grades in the fifth selection generation. It is possible to use the range of variation among the progeny of animals of these grades within the selection generation from which they are chosen to control the results of the cross. In Table IV[3] the first line represents the range of variation in the progeny of minus two animals of the sixth selection generation. The second line represents the range of variation in the progeny of plus three and one half and three and three quarters animals of the fifth selection generation. It will be seen that there is a space of seven grades between the two limits of variation of the parent races. The cross between these two types produced in the F_1 generation a range of variation shown in the third line of Table IV. It will be seen that for the most part, the young fall in the seven classes between the two parent races. In these seven classes will be found sixty-eight or 73.1 per cent. of the ninety-three young. Two or 2.27 per cent. of the young show grades of pig-

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[3] The references to tables and pages appearing in the first column of Table IV, and ensuing tables, refer to Castle and Phillips, 1914.

TABLE IV

Generation	−3	2¾	2½	2¼	2	1¾	1½	1¼	1	¾	½	−¼	0	+¼	½	¾	1	1¼	1½	1¾	2	2¼	2½	2¾	3	3¼	3½	3¾	4
−2 rats, Table 22, p. 41	—	—	3	22	324	368	119	60	51	14	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
+3½, +3¾ rats, Table 6, p. 34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	11	5	18	33	67	54	63	30	6
F_1, Table 50, p. 51	—	—	—	—	—	2	—	2	1	8	10	13	25	2	8	11	6	3	1	—	—	—	—	1	—	—	—	—	—
F_2, Table 50, p. 51	—	—	—	—	1	4	9	7	22	24	26	20	49	10	24	24	23	10	20	13	7	5	7	—	—	—	—	—	—

TABLE V

Generation	3½	3¼	−3	2¾	2½	2¼	2	1¾	1½	1¼	1	¾	½	−¼	0	+¼	½	¾	1	1¼	1½	1¾	2	2¼	2½	2¾	3	3¼	3½	3¾	4	4¼	4½
−2¾, −2⅞ rats, Table 26, p. 41	1	2	1	1	3	5	11	6	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
+3¾ rats, Tables 10 and 11, p. 36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	9	13	65	124	46	8	1
F_1, Table 50, p. 51	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	1	2	4	2	1	1	—	1	—	—	—	—	—	—	—	—	—
F_2, Table 50, p. 51	—	—	—	—	—	—	—	—	—	—	2	—	4	3	8	8	12	9	6	5	3	5	2	2	1	2	1	—	—	—	—	—	—

mentation characteristic of the plus parent and twenty-three or 24.75 per cent. of the young show grades characteristic of the minus parent race. When the F_1 young are bred *inter se* there is a marked increase in the per cent. of young showing grades of pigmentation characteristic of the grandparents. Ninety-three, or 30.5 per cent., of the 305 F_2 generation young fall in grades characteristic of the minus grandparents. Fifty-two, or 17.0 per cent., fall in grades characteristic of the plus grandparents. The remainder, or 52.5 per cent., fall in the seven intermediate classes.

A second cross made between selected animals is shown in Table V. Females of grade plus three and three quarters of the tenth selection generation were crossed with a male of grade minus three and one quarter of the tenth selection generation. As a control for the females it is possible to use the progeny of grade three and three quarter parents in the tenth and eleventh generations. The reason for using two generations instead of one is in order to increase the number of progeny available, since the tenth generation alone shows such a small number of young that they are not valuable as a significant breeding test. The range of variation in the progeny of rats of this grade is shown in the second line of Table V. As controls for the minus parent, rats of grade minus two and three quarters and two and seven eighths of the tenth selection generation have been used. Although the total of the young produced by them is very small (thirty-one), it is, nevertheless, the only critical data available. The range of variation of these young is shown in the first line of the table. The F_1 generation resulting from the cross of these two diverse selection types is shown in the third line of Table V. The thirteen young comprising this generation all fall in grades between the two parent types. The F_2 generation shows a distinctly greater variability than the F_1 generation and includes four young of grades characteristic of the plus grandparental race. This cross is less conclusive than the previous cross, but

TABLE VI

Generation	$3\frac{1}{4}$	3	$2\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{4}$	2	$1\frac{3}{4}$	$1\frac{1}{2}$	$1\frac{1}{4}$	1	$\frac{3}{4}$	$\frac{1}{2}$	$-\frac{1}{4}$	0	$+\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4	$4\frac{1}{4}$	$4\frac{1}{2}$	$4\frac{3}{4}$
$-1\frac{3}{4}$ Parents, Tables 17 and 18, p. 39	—	—	—	—	—	9	8	12	13	33	10	3	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 42, p. 48	—	—	—	—	—	—	—	2	—	4	6	5	6	13	2	1	3	4	5	3	4	2	1	—	—	—	—	1	—	—	—	—	—
−2 Parents, Table 22, p. 41	—	—	—	3	22	324	368	119	60	51	14	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 42, p. 48	—	—	—	—	—	—	1	1	1	1	2	6	6	7	2	3	6	4	2	4	1	—	—	—	1	—	—	—	—	—	—	—	—
$-2\frac{1}{8}$ Parents, Table 25, p. 42	1	—	4	22	78	251	100	15	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 42, p. 48	—	—	—	—	—	1	3	3	1	10	3	9	8	14	—	4	7	8	1	4	3	5	2	4	1	—	—	—	—	—	—	—	—
+3 Parents, Table 4, p. 33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	7	12	25	19	35	29	9	4	1	—	—	—	—
F_2, Table 42, p. 48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	5	3	2	3	4	2	1	—	—	—	—	—
$+3\frac{1}{4}$ Parents, Table 6, p. 34; $+3\frac{1}{2}$ Parents, Table 7, p. 35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	9	12	17	47	62	59	59	49	4	1	—	—
F_2, Table 42, p. 48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	1	1	1	6	9	8	8	1	—	—	—	—
$+4\frac{1}{4}$ Parents, Table 11, p. 36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	13	5	3	1	1
F_2, Table 42, p. 48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	3	4	2	3	3	—	—	—	—

TABLE VII

Generation	$-2\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{4}$	2	$1\frac{3}{4}$	$1\frac{1}{2}$	$1\frac{1}{4}$	1	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	0	$+\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$
$-1\frac{1}{2}$ × Parents, Table 20, p. 40; Table 19, p. 39	—	1	—	13	23	15	17	16	16	5	3	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 43, p. 48	—	—	—	1	5	3	6	12	26	9	9	10	1	3	2	2	1	—	—	—	—	—	—	—	—	—	—
$-1\frac{7}{8}$ Parents, Table 20, p. 40	—	—	6	31	50	29	12	11	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 43, p. 48	—	—	—	—	6	3	4	5	9	14	8	1	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—
−2 Parents, Tables 23 and 24, p. 41	7	19	76	843	818	158	26	51	9	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 43, p. 48	—	—	—	6	6	12	11	8	3	4	4	6	1	3	—	—	—	—	1	1	—	—	—	—	—	—	—
$+2\frac{1}{4}$ Parents, Table 2, p. 32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	3	1	1	—	1	—	—	—	—
F_2, Table 43, p. 48	—	—	—	—	—	—	—	2	4	5	4	16	7	16	22	25	29	24	13	33	14	8	8	6	3	—	—
+3 Parents, Table 4, p. 33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	7	12	25	19	35	29	9	4	1
F_2, Table 43, p. 48	—	—	—	—	—	—	—	2	—	—	—	1	1	3	3	4	2	2	1	2	1	1	—	—	—	—	—

does nevertheless show that 5.4 per cent. of the seventy-three F_2 young reproduce the degree of spotting characterizing one of the parental races.

The total number of animals observed, however, is considerably smaller than in the previous experiment and the results are therefore subject to greater error.

We may now consider several crosses made between hooded and wild rats. The F_1 generation in such crosses consists almost entirely of self pigmented animals. It is, therefore, in a comparison of the range of variability observed in the F_2 generation with that observed in the pure hooded race from which the hooded grandparent was taken, that we may expect to find evidence for or against segregation. The first cross to be recorded is between a female of grade $-1\frac{7}{8}$ in the $2\frac{1}{2}$ generation of the minus selection series, and a wild male. As a control for this female, the progeny of $-1\frac{3}{4}$ parents in the second and third selection generations may be taken. It will be seen that their progeny varies between grades -2 and $-\frac{1}{4}$ (see line 1, Table VI), in a total of 90 animals. The F_2 generation shows a range of variability between grades $-1\frac{1}{2}$ and grade $+3\frac{1}{2}$. It is interesting to note, however, that twenty-three or 37 per cent. of the sixty-two animals observed fall within the range of variation of the hooded grandparent. A comparison of grades $-1\frac{1}{2}$ and -2 in Fig. 2 will show how closely F_2 animals approach the extreme condition of the hooded grandparental race.

In the second cross between hooded and wild rats, females of minus two grade of the sixth generation of the minus selection series were used. As controls for these females the progeny of -2 parents in the sixth generation of the minus selection experiment may be used (see line 3, Table VI). The 969 young so obtained fall between the grades $-2\frac{1}{2}$ and $-\frac{1}{2}$. The forty-eight young show a large range of variability from $-1\frac{3}{4}$ to $+2\frac{3}{4}$. Of the forty-eight obtained, twelve, or 25 per cent., fall within the range of variation of the progeny of the hooded grandparental generation.

The third cross was between wild males and females of grade -2 or $2\frac{1}{4}$ in the tenth generation of the minus selection series. As a control for these animals it is possible to use the progeny of $2\frac{1}{8}$ parents in the tenth generation of the minus selection series (see line 5, Table VI). The 474 young so obtained vary between grades $-3\frac{1}{4}$ and -1.

Among the ninety-one F_2 young obtained, eighteen, or approximately 20 per cent., fall within the limits of variability of the hooded grandparents. The range of variability in the F_2 generation is considerable, being from -2 on one side to $+2\frac{3}{4}$ on the other.

Three crosses between animals of the plus selection series and wild rats are recorded. In the first cross (see lines 7 and 8, Table VI) females of grade plus three of the third generation of the plus selection series were used. The breeding capacity of these animals may be fairly judged by considering the progeny obtained from plus three parents in the third generation of the plus selection experiment. The 143 young so obtained range from grade $+\frac{3}{4}$ to $+3\frac{3}{4}$. Only twenty-one F_2 young are recorded, varying between grades $+1\frac{3}{4}$ and $+3\frac{1}{2}$. It is interesting to note that *all of them fall within the range of variation of the grandparental hooded race.*

In the second cross females of grade $+3\frac{1}{4}$, fifth generation and females of grade $+3\frac{1}{2}$, sixth generation, were crossed with wild males. The progeny obtained from animals of similar grade and generation within the selection experiment can serve as controls. The 320 young so obtained vary between grades $+1\frac{1}{2}$ and $+4\frac{1}{4}$. The thirty-eight F_2 young obtained vary between grades $+1\frac{1}{2}$ and $+3\frac{3}{4}$, again, in every case, reduplicating the grandparental forms.

The third cross, which includes only small numbers and is therefore of less relative value than the two previous crosses, was between a female of $+4\frac{1}{4}$, tenth generation and a wild male. The control animals gave twenty-five young ranging from grades $+3\frac{1}{2}$ to $+4\frac{3}{4}$. The F_2 generation, which consists of only sixteen animals, ranges between the grades $+2$ and $+3\frac{3}{4}$. Six of the sixteen young fall within grades characteristic of the grandparental animals (lines 11 and 12, Table VI).

Crosses between hooded and Irish rats have given comparable results (Table VII). Rats of grades $-1\frac{1}{2}$ generation $3\frac{1}{2}$ minus selection, were crossed with Irish rats. As a control for the hooded animals the progeny of rats of grade $-1\frac{1}{2}$ in the third and fourth selection generation may be used. The total of the progeny so obtained is 112. It will be noticed from Table VII, line 1, that they vary between grades $-2\frac{1}{2}$ and $+\frac{1}{4}$. Only eight or 8.8 per cent. of the F_2 generation vary *outside* of the grandparental grades. The range of variability in this generation is between -2 and $+1\frac{1}{4}$. The second cross is between females of grade $-1\frac{7}{8}$, from the fifth selection generation of the minus series and Irish males (lines 3 and 4, Table VII). As a control for these the progeny of $-1\frac{7}{8}$ parents of a similar generation may be used. The 143 progeny so obtained fall within the grades $-2\frac{1}{4}$ and $-\frac{3}{4}$ inclusive. The fifty-three F_2 young range between grades $-1\frac{3}{4}$ and $+1$. Almost exactly 50 per cent. of them reproduced the grades of the hooded grandparental types.

The third cross is between females of minus two grade in generation $7\frac{1}{2}$ of the minus selection experiment and Irish males. As a control, the progeny obtained from the females of -2 grade in the seventh and eighth selection generations may be used. Such animals produced 2,013 young, ranging from $-2\frac{3}{4}$ to $-\frac{1}{2}$. Sixty-six F_2 young ranged from grades -2 to $+2$. Of these, 75.8 per cent. reproduced the grades characteristic of the hooded grandparental race.

Two experiments are recorded showing crosses of plus selection animals with Irish males. In the first of these, the females used were of grade $+2\frac{1}{4}$ in the second generation of the plus selection experiment. Animals of similar grade and generation may act as controls, although the number of young obtained from them is very small.

The eight young so obtained fall between grades + 1¼ and + 2¾. From this cross 239 F_2 young have been obtained ranging from grades − 1 to + 3¼. 129 or 53.9 per cent. show grades characteristic of the hooded grandparent.

The remaining cross was between females of grade + 3, third selection generation and Irish males. Controls obtained by tabulating the progeny of animals of similar grade and generation show in 143 young, a range between grades + ¾ and + 3¾. Only twenty-three F_2 young were obtained, ranging between − 1 and + 2½. Of these, sixteen, or 69.5 per cent., reproduce the grades characterizing the hooded grandparent.

One other striking cross is recorded by Castle in the case of the rats. This is a cross between "mutant" rats showing a particularly advanced degree of plus pigmentation and pure wild rats. The range of variability of the pure "mutant" race is according to Castle's statement (Castle and Wright, 1916, page 173) between grades + 5¼ and + 5¾, see Table VIII. The 109 F_2 "mutant" young show a range of variation between the same grades. According to Castle's statement on page 174, "their range of variation does not fall beyond that of the uncrossed mutant race." It would appear as though the evidence of segregation in this case, even to an almost exact degree, was clear. This case, together with that of the type of spotting in mice known as "blaze" (Little, 1914) already discussed, appears to show that races differing from each other in only a minute degree of a quantitative character may show segregation clearly.

TABLE VIII

Generation	+5	5¼	5½	5¾	5⅞
Pure mutants	2	4	28	17	—
F_2 mutant × wild	1	6	49	50	3

To sum up the experiments with rats, it may be said that while it can not be claimed that the evidence is final in regard to the unit nature of the factors involved, they offer distinct evidence of segregation. That is to say, the combination of gametes formed by hybrid parents reproduce the same zygotic types as did combination of gametes of the pure parent races.

The bearing of the results on the practical breeding of farm animals seems clear. If a complicated and highly variable character as the hooded pattern in rats may be introduced in a cross with a non-hooded form and may be recovered in a large proportion of the F_2 generation, we may encourage crossing as a favorable method of producing new and important breeds. This will be all the more apparent if we agree with the selectionists who hold that the character reappearing in F_2 will be at once amenable to selection and improvement in a desired direction. *Contamination of genes* in breeding experiments which are conducted on a large scale and are followed by rigid selection, need not be considered as a factor of prime importance.

III. Physiological Factors Underlying Growth of Implanted Tissue

The study of the inheritance of spotting in mice and rats has served to give a considerable amount of data more or less directly comparable to that obtained in studies of size inheritance; though presenting, as I shall try to show, certain advantages in their freedom from environmental and age influences and in their definiteness.

We may now consider a very different line of work, which bears a most interesting relation to other studies of the inheritance of complicated morphological and physiological characters. The reaction of various closely inbred strains of mice and their hybrids to implants of a single tumor is definite, and characteristic. It is moreover undoubtedly hereditary as the work of many investigators has shown.

Tyzzer and the writer (1916) have reported results obtained in inoculating Japanese waltzing mice, closely inbred races of common mice and their hybrids, with an epithelial tumor which originated in the Japanese waltzing race. The parent animals all of them came from races which, because of long continued inbreeding, may be considered to have reached a degree of great genetic homogeneity. It will be seen from the table (loc. cit., p. 403) that all the Japanese waltzing mice inoculated, 58 in number, grew the implanted tumor, while none of the common mice showed continuous growth of the tumor. This absolute difference between the parent races is extremely interesting and offers ideal material for the formation of intermediate conditions of susceptibility in hybrid generations. They further offer a test of the relative value of the hypotheses of multiple factor and of blending inheritance. Sixty-two F_1 hybrids obtained by crossing the Japanese with tame races were inoculated. Of these, sixty-one grew tumors. The tumors grew in most cases fully as rapidly if not more rapidly than in the susceptible Japanese parent races. It is probable that the one animal in this generation which failed to grow the tumor is not a true exception, but that its behavior may be due to poor technique. The second generation hybrids have given an extremely interesting result. Of the 183 inoculated, only three have shown continuous growth of the tumor. This result is surprising in view of the fact that susceptibility appeared to be a dominant character in the F_1 generation. Only thirty-eight animals of the F_3 generation were inoculated; none of them grew the tumor. The striking difference between the F_1 and the F_2 generations suggests at once, alternative rather than blending inheritance. As we have suggested in our previous paper, the most logical interpretation appears to be that a certain physiological condition on which the growth of the tumor depends, is produced in the animals of the Japanese waltzing race. This condition is not found in the tame mice used. The differences between the races are hereditary, since succeeding generations of the Japanese and tame mice behave like their parents. The fact that susceptibility of the tumor occurs in both F_1 and

F_2 generation hybrids shows that the conditions on which the growth of the tumor depends are reproduced in hybrids of the two races. The behavior of the F_1 hybrid generation produced by reciprocal crosses indicates clearly that even when only one of the parents is susceptible and comes from the homogeneous Japanese race, its contribution to its offspring is sufficiently powerful to produce susceptibility in that animal. In other words, we may say that the hereditary factor or factors underlying susceptibility are functional even when present in a "single dose." If there is a single general factor underlying susceptibility we should expect that the F_2 generation would show a large number of susceptible animals. This is not the case. It is possible, however, to consider the behavior of the F_1 generation as being largely due to heterozygosis and not to true inheritance. To eliminate this possibility a back-cross generation was made between F_1 animals and pure Japanese. The sixty-three animals comprising this generation all proved susceptible to the tumor and in a majority of cases grew it as rapidly as did the F_1 hybrids themselves. On the other hand, the F_1 generation crossed with tame mice gave seventy-eight young, all of which were non-susceptible.

In discussing the results, it was further suggested that the explanation which best fitted the facts, indeed the only explanation which fits all of the facts, is that susceptibility depends for its manifestation upon the simultaneous presence of several factors in either the homozygous or the heterozygous condition. The gametes of the Japanese race possess all or nearly all of these in a homozygous condition and therefore produce susceptible animals. The F_1 hybrids possess all of these factors in a "single dose"; they having been contributed by the Japanese parent, and are therefore susceptible. *When, however, the F_1 animals form gametes, these factors, if they are mendelizing and not blending in nature, will be distributed at random in the gametes.* The result will be that the larger the number of factors involved, the rarer will be the inclusion of all of them within a single gamete formed by the F_1 animals. Since susceptibility in the F_2 generation will depend upon the presence of all of the factors ordinarily found in the Japanese race, it follows that the greater the number of factors involved, the rarer will be the appearance of a susceptible animal in the F_2 generation. It further follows that the susceptible animals of the F_2 generation probably will not possess the factors in a homozygous condition, as did the Japanese grandparents, and therefore they will not, in most cases, breed true, as did the Japanese grandparents, to the character of susceptibility.

For a more detailed discussion of these results from a genetic point of view, the reader is referred to Tyzzer and the writer's earlier paper. It will suffice at the present time to emphasize the fact that it is the inherent nature of the tissue of the host animal that is being studied. The tumor itself is as near a biologic constant as one can obtain. Variation in its growth therefore means variation in the attitude towards it, taken by the host tissue. This attitude appears to be dependent upon a complex of distinct factors. If a change or substitution is made in any one of the members of this complex, a different reaction is obtained. The behavior of the different factors in any such complex is distinctly that of independent units in inheritance. The fact that the reactions of susceptibility and non-susceptibility are dependent on multiple factors seems established. If the tissue of the adult mouse may be analyzed in this way, the conclusion is far reaching. If the reaction of the tissue depends on its substance, and its substance depends in turn on a certain hereditary complex of factors, it is logical to suppose that the rate and extent of development of the tissue as well as other processes of significance to the organism depend, in so far as they are hereditary, on similar complexes of genetic factors. Environment undoubtedly influences certain characters in their development far more than others, and in this respect size appears to be one of the most susceptible.

On the other hand, rate and extent of growth is undoubtedly chiefly dependent on the nature of the tissue involved, and as we have seen there is every reason to believe that this depends on the interactions of a complex of genetic factors which are independent of each other in inheritance.

OTHER EXPERIMENTS WITH INOCULATED TUMORS IN MICE

Loeb and Fleisher (1912) have reported a series of investigations on the hereditary factors underlying the susceptibility of mice to a transplantable carcinoma. As parent stocks they used three races of mice, one American race, and two European. By breeding tests including several generations the percentage of American mice to show continued growth of the inoculated tumor was found to be eighty-four, while those of the European races I and II were twenty-three and three per cent., respectively. The F_1 generation between American and European I gave sixty-eight per cent. susceptible, F_2 from this same cross gave thirty per cent. susceptible. When American mice were crossed with European II an F_1 generation was obtained in which one hundred per cent. of the animals inoculated were susceptible. Only fourteen animals were tested and the number is too small to establish this as an accurate percentage for this generation. The F_2 generation of this cross gave twenty-six per cent. susceptible.

It is interesting to note that in the F_1 generation, where sufficient numbers were obtained to afford critical evidence, the percentage of susceptibility was intermediate between those of the parent races. There is a marked decrease in degree of susceptibility in the F_2 generation. The fact that some of the animals of the American parent race failed to grow the tumors shows that this race is in all probability not homogeneous, and the same is true of the European races since animals within a single race fail to react similarly to pieces of biologically similar tumor. If such is the case, we should expect an intermediate result in the F_1 generation, just as we do when we cross two races differing in size. The F_2 generation should also be intermediate, though *on the basis of blending inheritance we should expect the percentage of positive animals to be much closer to that observed in the F_1 generation than it actually is.*

On an hypothesis of multiple factors underlying susceptibility or immunity to the inoculated tumor the experimental results may well be explained. The F_3 generations of Loeb and Fleisher's work give a result further indicating the possible presence of multiple factors. If a large number of F_2 animals mated *inter se* at random are used to produce the F_3 generation the percentage of susceptible animals in F_2 should be roughly approached in the F_3 generation. 122 animals comprising the F_3 generation show twenty-four per cent. susceptibility as compared with thirty per cent. in the F_2 generation of the same cross. In the F_3 generation of the American times European II cross, sixty-six animals have been inoculated and have given only two per cent. of susceptibility. This difference is possibly due to the fact that F_2 animals forming gametes each closely resembling those of the grandparent European race were unconsciously chosen as parents for this generation.

The interesting part of Loeb's work is the fact that the relative homogeneity of the races of mice which he used approximate closely the conditions in respect to suceptibility and non-susceptibility which one ordinarily is dealing with in size crosses as, for example, Castle's work and also MacDowell's work with rabbits. In none of these cases has there been excessively close inbreeding in either parent race before crosses were made. There is, therefore, no definite complex of factors characterizing the race. As a result the percentage of susceptible animals varies and depends on the character of the particular animals used for breeding. The whole effect produced is to obscure the true nature of the processes involved. In respect to homogeneity the material at the disposal of Tyzzer and the writer possessed a great advantage which became apparent in the definite results produced.

Size inheritance studies have not been recently made with mammals of known ancestry and of approximately pure races. This fact greatly diminishes the value of the results obtained even though they represent work of the most painstaking sort. For this reason the writer started last January a series of experiments on size inheritance in pure races of rabbits. Polish rabbits are being used for the small parent and Flemish giant rabbits for the large parent. It is hoped by a careful study of variation within the pure races to understand more clearly the method of inheritance in the hybrid generations to be produced by later experiments.

To summarize the work with inoculable tumors, one may say that it presents a type of inheritance not explicable on an hypothesis of blending inheritance or of a single variable gene. All the results may, on the other hand, be satisfactorily explained by supposing that the nature of the host tissue and its reaction to the implant depend upon a complex of mendelizing factors.

CONCLUSION

The fact that three genetically distinct types of spotting exist in mice; that segregation of the degree of spotting occurs in both rats and mice; that segregation of minute quantitative characters like the "blaze" spotting in mice, and the pattern of the "mutant" rats occurs; and finally that the composition and reaction of epithelial tissue in mice depends upon a complex of mendelizing factors, all indicate that in mammals the multiple factor hypothesis is steadily being strengthened as a scientific theory and a practical principle of great interest and importance.

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